Distinct Response of Human B Cell Subpopulations in Recognition of an Innate Immune Signal, CpG DNA

Jaeho Jung, Ae-Kyung Yi, Xin Zhang, Jongseon Choe, Li Li and Yong Sung Choi

*J Immunol* 2002; 169:2368-2373; doi: 10.4049/jimmunol.169.5.2368
http://www.jimmunol.org/content/169/5/2368

**References**
This article cites 27 articles, 15 of which you can access for free at: http://www.jimmunol.org/content/169/5/2368.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Distinct Response of Human B Cell Subpopulations in Recognition of an Innate Immune Signal, CpG DNA

Jaeho Jung,† Ae-Kyung Yi,‡ Xin Zhang,* Jongseon Choe,‡ Li Li,* and Yong Sung Choi‡*‡

Innate immunity has recently gained renewed interest in its ability to regulate adaptive immunity. Among the innate immune signals, CpG DNA has revealed its potential as a vaccine adjuvant. However, the cellular mechanism for the effect of CpG DNA on the humoral immune response is not well understood. Here, we investigated the effects of CpG DNA on human B cell differentiation using highly purified B cell subsets: naive, germinal center (GC), and memory B cells. In the in vitro culture system that mimics the primary or secondary immune response in vivo, CpG DNA markedly augmented the proliferation and generation of plasma cells from naive and memory B cells. CpG DNA dramatically increased plasma cell generation from GC B cells. However, CpG DNA did not have effect on memory B cell generation from GC B cells. These results suggest that CpG DNA potentiates the B cell adaptive immune response by enhancing terminal differentiation, but does not affect the generation of memory B cells. *The Journal of Immunology, 2002, 169: 2368–2373.

In the humoral immune response to T cell-dependent Ag, B lymphocytes first proliferate in T cell-rich areas and develop locally into plasma cells (PC), whereas a minority migrates to follicles to form the germinal center (GC). GC B cells then undergo a complex set of events, which involves interactions with T cells and follicular dendritic cells (FDC) that ultimately results in the formation of Ag-specific memory B cells and Ab-secreting PC. Memory B cells upon secondary exposure to Ags develop rapidly into PC-secreting Abs with high affinity for Ag. Overall, the ability to produce adequate amount of Abs in a timely manner by B cells is important to cope with recurrent bacterial infections.

Bacterial DNA has been shown to stimulate various immune cells, including B cells, macrophages/monocytes, dendritic cells (DC), and NK cells, resulting in cellular activation and induction of polyclonal Ig and pro-inflammatory cytokines (1). The immunostimulatory effect of bacterial DNA is due to the presence of unmethylated CpG dinucleotides within specific flanking bases (CpG motif) (2). Its strong stimulatory effect on DC and B cells has indicated that this CpG motif of bacterial DNA or synthetic oligodeoxynucleotides (CpG DNA) may be a potent vaccine adjuvant. Recently an optimal human CpG motif has been identified, which is a 23-mer phosphorothioate oligodeoxynucleotide (S-ODN) with higher nuclease resistance than the phosphodiester equivalent (3). However, most studies of CpG DNA with B cells have been conducted with primary or unfractonated B cells, leaving the respective effects of CpG DNA on primary and secondary humoral immune responses unknown.

The induction of immunological memory is an important feature of vaccination. We thus asked whether the optimal human CpG DNA promotes the generation of memory B cells in the in vitro culture system that mimics the GC reaction in vivo (4). Unlike its potent effect on B cell proliferation and Ig production from primary (naive B cells) and secondary B cells (memory B cells), CpG DNA did not modulate the generation of memory B cells from GC B cells. However, CpG DNA markedly augmented PC generation from GC B cells. Our findings suggest that CpG DNA displays its adjuvant effect on the humoral immune response by enhancing terminal differentiation of B cells into PC, but not by increasing memory B cell generation.

Materials and Methods

**Oligodeoxynucleotides**

S-ODNs were purchased from Operon Technologies (Alameda, CA). The following S-ODNs were used: CpG DNA (2006, TCGTCGTTTTGZCGTTTTGCGTT), ZpG DNA (2117, TZGTTGTTTTGZTTTGTZGTT; Z: methylated cytosine) (3), and control DNA (GGTTGATGACTCAGCGCCGA; AP-1) (5). ODNs were purified as previously described (6) using pyrogen-free solutions and had undetectable levels of endotoxin by Limulus assay (Gen-Probe, San Diego, CA). S-ODNs (2 μg/ml) were used based on the maximum proliferating response of tonsilar B cells by CpG DNA.

**Antibodies**

The Abs used included the following: FITC-conjugated anti-CD20 (L27, IgG1; BD Biosciences, San Jose, CA), PE-conjugated anti-CD38 (HIT2, IgG1; BD Pharmingen, San Diego, CA), FITC-conjugated (Al 12-2; BD Pharmingen) or PE-conjugated isotype controls (Dako, Carpinteria, CA), unconjugated anti-CD38 (T16, IgG1; Immunotech, Westbrook, ME), anti-CD44 (NKI-P1, IgG1; Dr. C. G. Figdor, University Hospital Nijmegen, Nijmegen, The Netherlands), anti-IgD (H9, IgG1, Sigma, St. Louis, MO), unconjugated isotype control (IgG1, MOPC-21; BD Pharmingen), rat anti-mouse IgG1 microbeads (Miltenyi Biotec, Auburn, CA), and FITC-conjugated goat anti-mouse Ig (BD Pharmingen).

**Cytokines and reagents**

Soluble human CD40 ligand (CD40L) was provided by Dr. R. Armitage (Immunex, Seattle, WA). The mAb against human CD40 (G28.5) was obtained from Dr. J. A. Ledbetter (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA). Rabbit anti-human IgM immunoglobulins...
(anti-μ) were purchased from Bio-Rad (Hercules, CA), anti-Ig bead suspension was purchased from Bio-Rad (Richmond, CA). Percoll and Ficoll were purchased from Pharmacia LKB Biotechnology (Uppsala, Sweden), and BSA was obtained from Sigma. IL-2 was obtained from Hoffmann-La Roche (Nutley, NJ), IL-4 was purchased from Schering Plough (Union, NJ), and IL-10 was obtained from R&D Systems (Minneapolis, MN).

Preparation of HK cells and B cell subsets

HK cells were established as described previously (7). Tonsillar B cells were prepared as described previously (8). Naïve and memory B cells were purified from high density tonsillar B cells by magnetic cell separation (Miltenyi Biotec) (9). Their purity was >98% when analyzed by flow cytometry after staining with anti-IdD and anti-CD38. GC B cells were obtained from low density B cells by depleting CD44° cells (4). The purity of GC B cells was >98% by expressing CD20°CD18high.

Culture of B cell subsets with HK cells

The culture medium was IMDM (Irvine Scientific, Santa Ana, CA) supplemented with 10% FCS (Life Technologies, Grand Island, NY), 2 mM l-glutamine, 100 U/ml penicillin G, and 100 μg/ml streptomycin (Irvine Scientific). GC B cells or memory B cells were cultured with HK cells (2 x 10⁵ cells/well, irradiated with 3000 rad), CD40L (400 ng/ml), IL-2 (50 U/ml), IL-4 (50 U/ml), or IL-10 (30 μg/ml) for 5 days. The optimal concentrations of these reagents were determined by their ability to support the proliferation of high density B cells or GC B cells.

Proliferation assay

Tonsillar B cells (1 x 10⁵ cells/well) were cultured in triplicate for 72 h in 96-well flat-bottom microtiter plates with or without CpG DNA in the presence of IL-2 (50 U/ml), IL-4 (50 U/ml), and IL-10 (30 μg/ml) together with anti-μ (10 μg/ml) and/or anti-CD40 Ab (100 ng/ml). Cells were pulsed with 0.5 μCi [³H]thymidine (DuPont NEN, Boston, MA) during the last 16 h of culture period. The cultures were harvested onto glass-fiber filters, and [³H]thymidine incorporation was measured by a liquid scintillation counter (Rackbeta; LKB Instruments, Houston, TX).

Flow cytometry and ELISA

Cells were stained with a panel of Abs unconjugated or directly conjugated with PE or FITC as previously described (4). Briefly, cells were incubated with the appropriate concentration of Ab for 15 min at 4°C. Unconjugated mAbs were detected by FITC-labeled goat anti-mouse Ig. After washing with PBS containing 0.2% BSA and 0.1% sodium azide, cells were fixed with 1% paraformaldehyde and analyzed by FACS. Large HK cells were excluded during cytometric analysis according to the forward and side scatter parameters. Flow cytometric analysis was conducted on a FACS-Calibur (BD Biosciences) with CellQuest software. The amount of IgM or μ chain in the culture supernatant was measured by ELISA as described previously (10).

RT-PCR

RT-PCR was performed using cDNA from both HK and GC B cells with primers specific for Toll-like receptor 9 (TLR9) and GAPDH. Total RNAs from HK and GC B cells were isolated using Purescript (Gentra System, Minneapolis, MN) according to the manufacturer’s instructions. Isolated RNA (1 μg from each sample) was treated with DNase (Life Technologies, Gaithersburg, MD) and then reverse transcribed with oligo(dT) primer using Superscript II reverse transcriptase (Moloney murine leukemia virus reverse transcriptase; Life Technologies). One tenth of the cDNA product was then amplified with the following gene-specific primers (5’ and 3’ primers, respectively): TLR9 (5’-AACACACAATCCAGAGCCA-3’ and 5’-AAGCCGAGTAAATTGTCAGG-3’), and GAPDH (5’-CCCTCCAAAAATCAATTGGGG-3′ and 5’-CGCCACAGTTTCCCGAGG-3’). Forty cycles of PCR were conducted with a temperature profile of 1 min at 94, 57, and 72°C. PCR products (250 bp for TLR9 and 347 bp for GAPDH) were separated by 2% agarose gel electrophoresis and visualized.

Results

CpG DNA augments activated B cell proliferation and PC generation from naïve B cells

To investigate the functional role of CpG DNA in the Ab response, we performed dose titration of CpG DNA to determine the optimum doses in various activating conditions of human B cells. As shown in Fig. 1, the optimum dose appears to be in the range of 0.5–2 μg/ml whether B cells were activated with anti-CD40 or anti-Ig. There was no significant donor variation (data not shown).

We investigated the effect of CpG on naïve B cells to study its function in the primary IgM Ab response. Highly purified IgD+CD20+CD38low naïve B cells were stimulated with CpG DNA in a 5-day culture system that contained IL-2 plus IL-4 or IL-2 plus IL-10 in addition to anti-μ and anti-CD40. These T cell-derived cytokines were chosen because IL-4 and IL-10 are well-known B cell growth and differentiation factors, respectively, for human naïve B cells, and the synergistic effect of IL-4 with either IL-4 or IL-10 is well established (11–13).

CpG DNA dramatically increased viable cell recovery in the cultures with IL-2 plus IL-4 (193 vs 753%) or with IL-2 plus IL-10 (67 vs 1055%; Fig. 2A). It also remarkably increased IgM secretion in the culture with IL-2 plus IL-10 (2.3 vs 40.2 μg/5 x 10⁵ cells; Fig. 2B). Although CpG DNA increased IgM secretion in the culture with IL-2 plus IL-4, considering the comparable cell recoveries (753 vs 1055%), the amount of IgM secreted in the culture with IL-2 plus IL-4 was much smaller than that with IL-2 plus IL-10 (6.8 vs 40.2 μg/5 x 10⁵ cells). To examine the specificity of CpG DNA, naïve B cells were cultured with ZpG DNA, which has the same sequence as CpG DNA except a substitution of cytosine with 5-methylcytosine, or with control DNA. ZpG DNA induced slight increases in viable cell recovery and IgM production in the culture containing IL-2 plus IL-10, while the addition of control DNA resulted in no significant change (Fig. 2, A and B). Neither viable cell recovery nor IgM secretion was significantly altered by ZpG DNA or control DNA in the culture with IL-2 and IL-4. These results indicate that CpG DNA enhances both IL-4- and IL-10-induced proliferation and IgM production of naïve B cells. The
enhanced response of cellular proliferation and IgM secretion was observed in similar experiments with three different donors.

The enhancing effect of CpG DNA on IgM secretion was substantiated by the phenotypic analysis of the cells harvested at the end of a 5-day culture period. The addition of CpG DNA increased CD20<sup>+</sup>CD38<sup>hi</sup> PC generation in the culture containing IL-2 plus IL-4 (1.7 ± 0.7 vs 5.0 ± 1.7%; p > 0.05; n = 3; Fig. 2C, left panels).

Taken together, these data suggest that in the primary immune response, CpG DNA expands the pool of Ag-specific B cells in the presence of IL-2 and IL-4 and enhances the generation of PC in the presence of IL-2 and IL-10.

CpG DNA enhances PC, but not memory B cell, generation from GC B cells

Using an in vitro experimental system that mimics GC reaction during the secondary immune responses in vivo, we have previously demonstrated that the FDC-like cell line, HK, and CD40L are essential for GC B cell growth and survival (4). At the same time, T cell-derived cytokines determine the differentiation pathway of GC B cells; CD20<sup>+</sup>CD38<sup>hi</sup> GC B cells differentiate into CD20<sup>+</sup>CD38<sup>lo</sup> memory B cells in the presence of IL-2 and IL-4 and into CD20<sup>+</sup>CD38<sup>hi</sup> PC in the presence of IL-2 and IL-10 (4, 14, 15).

FIGURE 2. CpG DNA markedly augments activated B cell proliferation and PC generation from naive B cells. IgD<sup>+</sup>CD20<sup>+</sup>CD38<sup>lo</sup> naive B cells were cultured with anti-CD40 Ab, anti-μ, and different cytokine combinations (IL-2 plus IL-4 vs IL-2 plus IL-10) in the presence or the absence of CpG DNA, ZpG DNA, or control DNA. Cells were washed after 3 days and were recultured for an additional 2 days under the same conditions. A, Viable cell recovery by trypan blue exclusion assay was calculated as a percentage of the initial number of viable cells. B, IgM production from culture supernatant was measured by IgM-specific ELISA. The results are the mean ± SD of triplicate cultures. C, Generation of CD20<sup>+</sup>CD38<sup>hi</sup> PC and CD20<sup>+</sup>CD38<sup>lo</sup> memory B cells in the presence or the absence of CpG DNA was examined by flow cytometry after staining with anti-CD20 and anti-CD38 mAbs. Numbers represent the frequency of CD20<sup>+</sup>CD38<sup>hi</sup> PC. The experiment was repeated three times with similar results.

Enhanced immune response of cellular proliferation and IgM secretion was observed in similar experiments with three different donors.

FIGURE 3. CpG DNA dramatically increases PC generation, but not memory B cell generation, from GC B cells. CD20<sup>+</sup>CD38<sup>hi</sup> GC B cells were cultured with HK cells, CD40L, and different cytokine combinations (IL-2 plus IL-4 vs IL-2 plus IL-10) in the presence or the absence of CpG DNA, ZpG DNA, or control DNA. Cells were washed after 3 days and were recultured for an additional 3 days under the same conditions.

A, Viable cell recovery by trypan blue exclusion assay was calculated as a percentage of the initial number of viable cells. B, IgG production from culture supernatant was measured by IgG-specific ELISA. The results are the mean ± SD of triplicate cultures. C, Generation of CD20<sup>+</sup>CD38<sup>hi</sup> PC and CD20<sup>+</sup>CD38<sup>lo</sup> memory B cells in the presence or the absence of CpG DNA was examined by flow cytometry after staining with anti-CD20 and anti-CD38 mAbs. Numbers represent the frequency of CD20<sup>+</sup>CD38<sup>hi</sup> PC. The experiment was repeated with similar results.
To explore the effect of CpG DNA on the proliferation and differentiation of GC B cells, we cultured CD20+/CD38hi GC B cells for 6 days in the presence or the absence of CpG DNA. Unlike naive B cells, CpG DNA did not have an effect on GC B cell recovery in the presence of IL-2 and IL-4 (283 vs 252%; Fig. 3A). CpG DNA synergizes with B cell receptor stimulation (2). However, CpG DNA, even with the signals delivered through the Ag receptor, did not affect the recovery (data not shown). The cells recovered at the end of a 6-day culture with IL-2 plus IL-4 were memory B cells, as indicated by the induction of CD44 expression (16, 17) (data not shown). The addition of CpG DNA to the culture containing IL-2 and IL-10, however, resulted in a 5-fold increase in viable cell recovery (310 vs 1530%; Fig. 3A). There was a remarkable increase in IgG production in the presence of CpG DNA (11 vs 39 μg/2 × 10⁵ cells; Fig. 3B). The control ZpG DNA had modest effects, while control DNA did not modulate either viable cell recovery or IgG production (Fig. 3, A and B). Flow cytometric analysis revealed that enhanced IgG secretion by CpG DNA resulted from the increased generation of CD20+CD38hi PC from CD20+CD38hi GC B cells. The absolute number of PC was increased by 5.5-fold when CpG DNA was present in the culture (39 vs 223 × 10⁴ cells; Fig. 3C).

Since the culture system for GC B cells contained HK cells, it was necessary to determine whether the effect of CpG DNA on GC B cells was direct or indirect through HK cells. When HK cells were pulse-treated with CpG DNA before coculture with GC B cells, there was no enhancing effect of PC generation (data not shown). We also measured the expression levels of TLR9, a known CpG DNA receptor (18). RT-PCR analysis revealed that GC B cells, but not HK cells, express TLR9 mRNA (Fig. 4). Therefore, CpG DNA appears to enhance the generation of PC by acting directly on GC B cells.

Response of memory B cells to CpG DNA is different from that of GC B cells

Memory B cells are generated in GC of secondary lymphoid organs (19). They either undergo terminal differentiation into PC or re-enter GC for accumulation of somatic hypermutation (20). Since CpG DNA did not have any enhancing effect on the generation of memory B cells from GC B cells (Fig. 3A), and generation and re-activation of memory B cells are different processes, we investigated the effect of CpG DNA on the proliferation and differentiation of memory B cells freshly isolated from tonsil.

IgD−CD20+CD38low memory B cells were cultured with either IL-2 plus IL-4 or IL-2 plus IL-10 in the culture system containing HK cells and CD40L for 6 days. In the culture containing IL-2 plus IL-4, memory B cells maintained their phenotype of CD20+CD38lo (Fig. 5A). CpG DNA enhanced the proliferation of these cells, resulting in an increase in viable cell recovery from 248 to 563% (Fig. 5B). The presence of IL-2 and IL-10, in contrast, directed memory B cells to differentiate into CD20+CD38hi PC (Fig. 5A). The addition of CpG DNA gave rise to an enhanced recovery of viable cells from 962 to 2135% (Fig. 5B) accompanied by significantly increased IgG secretion in the culture supernatants.

**FIGURE 4.** The CpG DNA receptor, TLR9, is expressed in GC B cells, but not in HK cells. GC B cells and HK cells were analyzed for TLR9 and GAPDH mRNA expression by RT-PCR as described in Materials and Methods. The results shown are representative of three experiments.

**FIGURE 5.** CpG DNA enhances cellular proliferation and PC generation from memory B cells. IgD−CD20+CD38low memory B cells were cultured with HK cells, CD40L, and different cytokine combinations (IL-2 plus IL-4 vs IL-2 plus IL-10) in the presence of the absence of CpG DNA. Cells were washed after 3 days and were recultured for an additional 3 days under the same conditions. A, The phenotype of cultured memory B cells was examined by flow cytometry after staining with anti-CD20 and anti-CD38 mAbs. B, The percentage of viable cell recoveries under the culture conditions containing IL-2 plus IL-4 or IL-2 plus IL-10 was calculated by trypan blue exclusion as described in Fig. 2A. C, The supernatants in the cultures containing IL-2 plus IL-10 were harvested, and IgG concentrations were measured by IgG-specific ELISA. The results are the mean ± SD of triplicate cultures. The experiment was repeated twice with similar results.
Bacterial DNA enhanced PC generation from GC B cells

Since the above experiments were performed with synthetic oligonucleotides, we performed the similar experiments with bacterial DNA to establish a physiological relevance in the CpG effect on the host defense mechanism. When different doses of Escherichia coli and calf thymus DNA were added to anti-IgM activated B cells, E. coli DNA increased cellular proliferation in a dose-dependent manner, whereas the control mammalian DNA (e.g., calf thymus) did not increase (Fig. 6). These data suggest that bacterial DNA stimulates anti-IgM-activated B cells, similar to that observed with oligonucleotides.

We next repeated similar experiments with GC B cells as described above. GC B cells purified from tonsillar B cells were cultured in various concentrations of E. coli DNA and calf thymus DNA. As shown in Fig. 7, E. coli DNA did not enhance memory B cell proliferation compared with calf thymus DNA in the culture containing CD40L and IL-2 plus IL-4. In the culture containing IL-2 plus IL-10, E. coli DNA enhanced PC generation at all doses tested (0.25-4 μg/ml), whereas calf thymus DNA did not have a significant effect. This result was reproducible because a similar observation was made with GC B cells from two other donors.

Discussion

CpG DNA was first shown to directly trigger B cell activation, inducing cellular proliferation and Ig secretion in primary B cells or B cell lines. We have investigated the effect of CpG DNA on three major subpopulations of human B cells, naive, GC, and memory B cells, because the activation and differentiation requirements of these B cell subsets are different. We found that CpG DNA displayed a remarkable enhancing activity in PC generation from all B cell subpopulations. CpG DNA had no effect on memory B cell generation from GC B cells.

In the primary immune response, naive B cells undergo proliferation and differentiation along two different pathways following the Ag stimulation. B cells can either differentiate into IgM-secreting PC, thus providing a rapid source of neutralizing Abs with low affinity, or become GC B cells. When naive B cells were activated by anti-μ and T cell-derived cytokines, the condition that mimicked the primary immune response in vivo, CpG DNA significantly enhanced cellular proliferation. These proliferating cells can initiate GC formation or undergo the terminal differentiation into PC secreting IgM. In the presence of IL-10, CpG DNA enhanced PC generation dramatically, suggesting that CpG DNA first expands pathogen-reactive B cell pools and then promotes their differentiation into PC at the extrafollicular areas. This mechanism would provide an efficient means of initiating early protective immunity when a low dose of bacteria enters the body. This hypothesis is consistent with the recent report that polyclonal IgM is essential for the resistance of mice to a systemic bacterial infection (21).

Strong augmenting effects of CpG DNA on B cells have suggested that it can be used as an efficient vaccine adjuvant. Indeed, Davis et al. (22) showed that CpG DNA was a potent immune enhancer when mice were injected with recombinant hepatitis B surface Ag. It induced a remarkably increased IgG Ab response against the surface Ag. However, it is not clear how CpG DNA enhances the secondary immune response, which is an important feature of vaccination. In the secondary immune responses, GC reaction is pivotal to the production of specific Abs with high affinity and memory B cells. In GC, where Ag-activated B cells recycle through cellular proliferation in the presence of activated T cells and FDC, B cells have two fates: either to become memory B cells with a potential to produce high affinity Abs or to differentiate to PC. PC generated in the GC migrate to the bone marrow where they secrete Abs with high affinity. Our study shows that CpG DNA, in contrast to its effect on cellular proliferation of naive B cells, did not influence the cellular expansion of GC B cells leading to memory B cell generation, but significantly augmented PC generation. This result suggests that the immune system, through recognition of CpG DNA, maximizes the clearance of pathogens by producing large amounts of high affinity Abs. CpG DNA exhibits its selective or regulatory effect on B cell subpopulations in extrafollicular areas and in the GC.

![FIGURE 6.](Image 1) Dose titration curve of E. coli DNA and calf thymus DNA in tonsillar B cells. Unfractionated tonsillar B cells (1 x 10^6 cells/well) were cultured for 3 days with various concentrations of E. coli DNA (EC-DNA) and calf thymus DNA (CT-DNA) together with cytokines (IL-2 and IL-10) in the presence of anti-IgM. Cells were pulsed for the last 16 h, and [3H]thymidine incorporation was determined. Results are expressed as the mean counts per minute ± SD of triplicate cultures.

![FIGURE 7.](Image 2) E. coli DNA has a similar effect on GC B cell differentiation as CpG DNA, but not calf thymus DNA. CD20^+CD38^{high} GC B cells were cultured with HK cells, CD40L, and IL-2 plus IL-4 (A) or IL-2 plus IL-10 (B and C) in the presence of various dose of E. coli DNA and calf thymus DNA. Cells were washed after 3 days and were recultured for an additional 3 days under the same conditions. A and B, Viable cell number was counted by trypan blue exclusion as described in Fig. 2A. C, IgG production in the culture supernatant of GC B cells was measured by IgG-specific ELISA. The results are the mean ± SD of triplicate cultures. A representative of three experiments is shown.

Downloaded from http://www.jimmunol.org/ by guest on September 15, 2017
CpG DNA augments the proliferation of ex vivo memory B cells as it does of naive B cells to expand the cellular pool. This would result in a further increased number of effector cells with much higher affinity for the re-encountered Ag. In addition, CpG DNA significantly increased PC generation from memory B cells. Therefore, our observation that CpG DNA increases both cellular proliferation and IgG secretion of memory B cells appears to re-inforce the characteristic prompt and robust Ab production during the secondary immune response. Furthermore, our findings on the effects of CpG DNA on resting B cells (naive and memory) are in line with a recent report about the increased proliferation of peripheral blood B cells after CpG DNA stimulation (5).

Of note, there was a modest, but significant, enhanced response of cellular proliferation and Ig production by ZpG DNA. The phosphorothioate backbone has been reported to induce moderate stimulation of human B cells in a CpG-independent manner (23). However, at 2 μg/ml no significant stimulation by ODN with phosphorothioate backbone (control DNA) was found, suggesting that the observed synergistic effect by ZpG DNA was not due to the phosphorothioate backbone. The moderate stimulatory effect, especially with regard to the culture containing IL-2 and IL-10, may be explained by the non-specific effects of the methylated CpG motifs as observed by other investigators (24).

Despite its promising clinical use as an adjuvant, the molecular mechanism by which CpG DNA activates immune cells remains less well defined. A recent study by Takeshita et al. (25) showed that the CpG DNA response of PBMC correlates with TLR9 expression. Krug et al. (26) also showed that TLR expression on DC of different origins was required for the recognition to innate immune signals such as CpG DNA and LPS. Since GC B cells express TLR9, CpG DNA may act through this receptor in GC B cells. TLR9 appears to use the myeloid differentiation factor 88/IL-1R-associated kinase/TNFFR-associated factor 6 signaling pathway, which leads finally to the activation of NF-κB and stress kinase pathways. In the course of GC B cell differentiation, T cells in GC play pivotal roles by regulating cytokine expression. IL-4 is a growth factor for memory B cells, while IL-10 is essential for PC generation. Since cytokines regulate TLR gene expression (27), TLR9 expression could be changed during the GC B cell differentiation pathway. However, there was no change in TLR9 gene expression by IL-4 or IL-10 during GC B cell differentiation (data not shown), suggesting that regulation of the CpG DNA receptor may not be involved in the selective effect of CpG DNA in the differentiation pathway of GC B cells. Further comparative studies of the CpG DNA signaling pathway in naive, GC, and memory B cells must be performed to understand how B cells respond to CpG DNA at different stages of differentiation.

The comparative studies of synthetic phosphorothioate ODN and bacterial DNA in their immunostimulation effects have been reported previously (2, 3, 5, 6). However, we have undertaken similar studies to address the physiological significance of our experimental results with ODN. First, we determined the optimum dose of bacterial DNA in stimulating human B cells and then investigated its effect on the differentiation of GC B cells. Although it is difficult to access the CpG concentration in bacterial DNA, the result has essentially confirmed the experimental data obtained with ODN. Bacterial DNA enhanced GC B cell differentiation significantly more than did the calf thymus DNA used.

In conclusion, our findings suggest that CpG DNA potentiates the B cell adaptive immune response very efficiently in an appropriate cytokine milieu and reinforce the concept of CpG DNA as a promising and potent vaccine adjuvant.

References